Molecular Mechanisms of Magnetosome Formation

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Abstract

Magnetotactic bacteria are a diverse group of microorganisms with the ability to use geomagnetic fields for direction sensing. This unique feat is accomplished with the help of magnetosomes, nanometer-sized magnetic crystals surrounded by a lipid bilayer membrane and organized into chains via a dedicated cytoskeleton within the cell. Because of the special properties of these magnetic crystals, magnetotactic bacteria have been exploited for a variety of applications in diverse disciplines from geobiology to biotechnology. In addition, magnetosomes have served as a powerful model system for the study of biomineralization and cell biology in bacteria. This review focuses on recent advances in understanding the molecular mechanisms of magnetosome formation and magnetite biomineralization.
INTRODUCTION

Magnetotactic bacteria (MB) are a phylogenetically and morphologically diverse group of microorganisms that can align in and navigate along geomagnetic fields (1). Examination of an MB cell under a transmission electron microscope reveals the physical basis for this phenomenal behavior (Figure 1). Each MB is equipped with one or more chains of a specialized organelle, consisting of a 30–50 nm crystal of the iron oxide magnetite or the iron sulfide greigite surrounded by a lipid bilayer membrane (1). This chain is fixed within the cell allowing the bacterium to be passively aligned in external magnetic fields. In the 30 years since their serendipitous discovery by Blakemore (2), MB and magnetosomes have been at the center of a variety of interdisciplinary efforts aimed at characterizing and exploiting the magnetic properties of their crystals (1). During this time, a steady stream of review articles has chronicled the progress of research on these organisms (1, 3–10). The reader is directed to an Annual Review of Microbiology article by Blakemore (4) for a historical perspective on the discovery and early studies on the ecological distribution of these bacteria. More recently, Bazylinski & Frankel (1) have provided an in-depth review of MB and magnetosomes. Here, some of the most significant achievements in magnetosome research over the past few years are reviewed with a special emphasis on the cell biology of magnetosomes and molecular mechanisms controlling the formation of the organelle and biomineralization of magnetite.

MAGNETOTACTIC BACTERIA: DIVERSITY AND EVOLUTION

MB have been observed in a variety of freshwater and marine aquatic environments and belong to a wide range of phylogenetic groups, including the α-Proteobacteria, δ-Proteobacteria, γ-Proteobacteria, and the Nitrospira (11–15). To date no gram-positive MB or magnetotactic Archaea have been identified. The tactic behavior of MB is more accurately described as magneto-aerotaxis (rather than magnetotaxis) because their alignment in applied magnetic fields is passive and sensing of oxygen or redox gradients is believed to dictate their swimming direction (16). It has generally been accepted that magneto-aerotaxis provided MB with a selective advantage by simplifying their search for microaerobic environments (1). Consistent with this model, it has been quantitatively demonstrated that MB swim away from advancing oxygen gradients faster in the presence of a magnetic field than in its absence (17). However, the recent discovery of a novel species of MB with an unexpected swimming behavior has put this model of magneto-aerotaxis in doubt (18) (see the Sidebar).

Because MB do not occupy a distinct branch of the phylogenetic tree, they cannot
be readily identified in large-scale environmental surveys that rely on housekeeping genes, such as 16S ribosomal RNA, for assessment of microbial diversity. For instance, *Desulfovibrio magneticus* RS-1 would be classified as a sulfate-reducing δ-proteobacterium on the basis of its 16S rRNA sequence, but it is also a magnetotactic bacterium capable of magnetosome formation (13). The converse of this scenario has also been reported. Perchlorate-reducing bacteria belonging to the *Dechlorospirillum* group are closely related to the *Magnetospirillum* group but cannot form magnetosomes (19). For these reasons, most efforts to identify MB in the environment have relied on the use of magnetotactic behavior as a marker for the presence of these organisms. Although some recent studies have elegantly described the distribution of MB in specific environmental niches (14, 20), the broader presence of these organisms in nature and their impact on the geochemical cycling of iron is not fully understood.

The mechanism for the widespread appearance of magneto-aerotaxis across the phylogenetic tree is also not understood at the moment. The most likely explanation is that the genes responsible for production of magnetite and magnetosomes have been moved horizontally across various groups of the Bacteria. This idea has gained some momentum with the discovery of an unstable genomic island necessary for magnetosome formation (see below). However, it is also possible that the ability to form magnetosomes was ancestral to all Bacteria, although this ability was lost from most species but maintained in modern day MB. A third possibility is that magneto-aerotaxis developed multiple times across evolutionary time.

**MAGNETOSOMES: VARIATIONS AND APPLICATIONS**

The magnetosome is the intracellular structure that allows MB to orient in external magnetic fields. It consists of a chain of magnetite (and in some cases greigite) crystals,
each of which is surrounded by a lipid bilayer membrane (1). The most commonly studied MB have a single magnetosome chain consisting of 15–20 ~50 nm crystals of magnetite. However, within the vast diversity of MB discussed above, a wide range of magnetosome morphologies has been observed. These include MB with hundreds of magnetic particles per cell, those containing multiple chains, and those containing one or two large crystals of magnetite (21). There is also considerable variation in the shape of the crystals produced by MB, with morphologies ranging from rounded to bullet shaped (12, 22). In almost all cases, however, the magnetic crystals are chemically pure and are single-domain magnets, meaning that they hold their magnetic moments stably under ambient conditions (21). Interestingly, magnetosome-like structures and magnetic minerals have been found in eukaryotic organisms as well. Algae, fish, termites, pigeons, honeybees, and even humans have been shown to have magnetic particles that in some cases appear to participate in the direction-sensing behavior of the organism (23–25). The strict biological control over the properties of the inorganic minerals produced by MB is reminiscent of many other biomineralization systems, such as formation of silica shells by diatoms and tooth and bone formation by animals. Because magnetosome-like chains of magnetite survive for long periods of time in sediments, geobiologists have used bacterial magnetite as “magnetofossils” to survey the history of life in ancient rocks and even to search for life signatures in extraterrestrial samples (10, 26, 27). The combination of the above observations has led to the hypothesis by Kirschvink & Hagadorn (10) that magnetosomes might have been the ancestral biomineralization system exapted over time to accommodate the formation of the biominerals seen in eukaryotic systems. As such, MB holds promise as a relatively simple and genetically tractable model system for understanding fundamental evolutionary and mechanistic aspects of biomineralization. Many biotechnological and biomedical applications have also been proposed for magnetosomes, including their use as contrast agents for magnetic resonance imaging and as convenient systems for the purification of specific proteins and nucleic acids (28, 29). The bulk of the research on magnetosomes has been focused on exploiting and understanding the properties of the magnetic crystals formed within magnetosomes. However, recent work has shown that magnetosomes can also be an ideal model for the study of organelle development in bacteria (30–32).

**CELL BIOLOGY OF MAGNETOSOMES**

It is now appreciated that most bacteria have a highly organized subcellular architecture and use sophisticated mechanisms to control cell shape, cell division, and chromosome segregation (33). The discovery of dynamic bacterial cytoskeletal proteins related, and perhaps ancestral, to eukaryotic actin, tubulin, and intermediate filaments has shown that study of bacterial cell biology can lead to broader insights into the evolution of eukaryotic cell structure (34). Membranous organelles, once considered a hallmark of eukaryotic cells, have also been described for a variety of bacteria (35, 36). Magnetosomes are perhaps the best studied of these bacterial organelles and display many of the common features of eukaryotic organelles. A lipid bilayer membrane containing a distinct set of proteins surrounds each magnetite crystal, and magnetosomes are organized into chains via a dedicated cytoskeleton (30, 32, 37). In addition, a fully functional magnetosome membrane is present prior to magnetite formation and is the site of its biomineralization, showing that this membranous compartment behaves as an independent organelle (30, 31).

**Magnetosome Membrane**

Over the years, a combination of electron microscopic studies and lipid composition analyses of magnetosomes led to a model that
the magnetosome membrane was a lipid bilayer vesicle originating from the cell membrane (2, 38–40). Recently electron cryotomographic (ECT) imaging of magnetosomes in three dimensions provided an unprecedented view of the organelle and challenged parts of this widely accepted model for the subcellular organization of magnetosomes (30, 32). In ECT imaging, a series of two-dimensional images of a given specimen, collected at various angles relative to the electron beam, is algorithmically reconstructed into a three-dimensional image (41). ECT imaging of *Magnetospirillum magneticum* sp. AMB-1 (AMB-1) was used to explore the ultrastructure of MB in a near native state without disruptive staining, fixation, and sectioning procedures (Figure 2) (30). Many of the known features of magnetosomes, such as the presence of a membrane prior to magnetite biomineralization and the juxtaposition of the chain against the membrane, were confirmed in this study. Surprisingly, however, all clearly imaged magnetosomes in the ECT reconstructions were shown to be invaginations of the cell membrane regardless of their place in the chain or the size of the magnetite crystal within them, leading to the conclusion that magnetosomes are not vesicles (30). It is unclear if this feature is universal to all MB. ECT imaging of *Magnetospirillum gryphiswaldense* sp. MSR-1 (MSR-1) showed that the magnetosome chain was indeed close to the cell membrane, but the presence of membrane invaginations as not directly investigated (32).

These results raise several new questions regarding the biology of magnetosomes. First, organization of the magnetosome as a cell membrane invagination insures that magnetosome chains are connected to the cell, thus facilitating the alignment of MB in magnetic fields. Second, it has been hypothesized that ferrihydrite, an iron oxide mineral that is a precursor to magnetite, is formed in the periplasm and transported into the magnetosome, where it is then reduced to form magnetite (42). The opening of the magnetosome to the periplasm may be crucial for the transport of such an intermediate. Third, the presence of an open channel between the magnetosome and the periplasm provides an opportunity for the contents of the two compartments to mix, an event that may be disruptive to magnetite formation. Thus, it is likely that a barrier (not visualized through ECT imaging) exists between the two compartments or that specific proteins are used for the purification of the magnetosome lumen.

**Figure 2**

Three-dimensional organization of magnetosomes. An ECT reconstruction of *Magnetospirillum magneticum* sp. AMB-1. The background is the inner membrane, and magnetosomes can be seen as invaginations of the inner membrane. A network of cytoskeletal filaments surrounds the magnetosome chain. Image courtesy of Zhuo Li & Grant Jensen.
Finally, several modes of membrane bending have been described for eukaryotic cells, and it would be interesting to see if MB use either related proteins or similar mechanistic themes in forming magnetosomes (43).

**Magnetosome Chain Formation**

Another fascinating property of magnetosomes is their organization as chains within the cell. In theory, individual magnetite crystals produced in vitro would not tend to organize end to end into a long chain and instead should collapse into aggregates, implying the presence of specific mechanisms for stabilization of magnetosome chains. In fact, purified magnetosomes still retain their chain-like organization but end up as aggregates upon the removal of the magnetosome membrane and proteins through a detergent treatment (39, 40). ECT imaging studies of AMB-1 and MSR-1 have provided a clear view of the subcellular structures that appear to ensure the stability of the magnetosome chain. For both of these organisms, a network of filaments surrounds the magnetosome chain (Figure 2). These filaments have dimensions similar to bacterial actin-like proteins and might be formed by a magnetosome-specific cytoskeletal protein (see below) (30, 32).

The process of chain formation as cells transition from a nonmagnetic to a magnetic state has been investigated for some MB. In AMB-1, the growth of cells under iron-poor conditions does not affect cell growth but prevents the formation of magnetite. Interestingly, under these conditions, chains of empty magnetosome membranes are formed prior to the biomineralization of magnetite (30, 31). Upon addition of iron, magnetite production proceeds simultaneously within multiple adjacent magnetosomes until full-sized crystals have been formed (31). Cells of MSR-1 seem to use a different mechanism of forming a magnetic chain. First, magnetosomes are quite dispersed throughout the cell prior to magnetite formation, and as biomineralization proceeds, magnetic interactions seem to be required to align magnetosomes into a chain (32). These apparently distinct modes of chain formation might result from differences in growth conditions or may reflect fundamental differences in the molecular mechanisms of chain organization within the cell.

It is important to note that these studies follow the formation of a “magnetic” chain as cells transition from iron-poor to iron-rich conditions and that the control of chain formation during the normal growth of the cells is not well understood. Individual MB cells within a population have a relatively constant number of magnetosomes per chain, and the chain is seen to separate down the middle during cell division. Therefore all cells need to double the number of magnetosomes during each cell cycle. Preliminary attempts at examining magnetosome dynamics during synchronized growth of AMB-1 cells have been reported, but at the moment, it is unclear if the spatial or temporal production of magnetosomes and their assembly into the chain are cell cycle regulated (44).

**MAGNETOSOME GENES**

Progress in the various aspects of magnetosome research, be it their use in biotechnological applications or as a model system for the study of bacterial cell biology, has been greatly limited by a substantial gap in the understanding of the molecular pathways leading to the formation of magnetosomes. As detailed below, the recent convergence of genetic, proteomic, and genomic approaches has led to the identification of a large genomic island containing many of the factors required for magnetosome formation.

**Model Organisms**

One of the main obstacles in the molecular study of MB had been the lack of reliable model systems for genetic and biochemical work. Two different species of MB, AMB-1 and MSR-1, are now established systems for molecular analysis (31, 45, 46). In addition,
the genome sequences of several MB are now available. These include the partial genome sequences of *Magnetospirillum magnetotacticum* sp. MS-1 ([http://genome.jgi-psf.org/draft_microbes/magma/magma.home.html](http://genome.jgi-psf.org/draft_microbes/magma/magma.home.html)) and *Magnetococcus* sp. MC-1 ([http://genome.jgi-psf.org/draft_microbes/magm1/magm1.home.html](http://genome.jgi-psf.org/draft_microbes/magm1/magm1.home.html)), advanced efforts in finishing the MSR-1 genome, and the complete and fully assembled genome sequence of AMB-1 (47). These genomes have been a powerful tool for comparative analysis of magnetosome gene clusters (see below) and have allowed for genome-wide analysis of transcription (48, 49). They have also revealed interesting features of MB that may lead to a better understanding of the evolution and function of magneto-aerotaxis. For instance, the sequenced species of the *Magnetospirilla* contain some of the highest numbers of signaling-related genes among all bacteria, including over 60 homologs of methyl-accepting chemotaxis proteins (as a comparison, *E. coli* contains only 5 of these proteins) (50, 51). Whether there is a connection between this unusually large catalog of signaling genes and magneto-aerotaxis has yet to be determined.

### Identification of Magnetosome Genes

The first major breakthrough in identifying magnetosome genes came through efforts to purify magnetosome-specific proteins. Isolation of magnetosomes is quite simple. Once a cell extract has been prepared, magnetosomes can be easily separated with a magnet (39, 40). This technique allowed Gorby et al. (39) to demonstrate that the purified magnetosome fraction had a distinct pattern of proteins compared to other cellular fractions. Subsequent work from other groups revealed the identity of several of these magnetosome-specific proteins from *Magnetospirillum magnetotacticum* sp. MS-1, AMB-1 and MSR-1 (40, 52–55). Notably, Grunberg et al. (55) showed that several magnetosome proteins (MamA, MamB, MamC, MamD, and MamE) were organized into two discrete gene clusters found in all MB with available genome sequences. The first of these is predicted to contain 17 genes and includes the *mamA*, *mamB*, and *mamE* genes, and the second group is part of a 4-gene cluster, containing *mamC* and *mamD* (55). Large-scale proteomic work with MSR-1 further confirmed the association of these proteins with magnetosomes, showing that 12 of the 17 genes within the *mamAB* cluster, all of the genes of the *mamCD* cluster, and the genes of a third group (the *mms6* cluster) encode proteins that localize to the magnetosome (40). Proteomic analysis of AMB-1 has also shown that several of the *mamAB*, *mamCD*, and *mms6* gene products are enriched on the magnetosomes of this organism (51). The importance of these magnetosome-specific proteins in magnetosome formation has been demonstrated through genetics. Transposon insertions and directed deletion of genes in the *mamAB* cluster lead to a range of defects in formation of magnetite and assembly of a magnetosome chain (30–32). Furthermore, spontaneous mutants of both MSR-1 and AMB-1, lacking a large (~100-kb) region of the genome that includes the *mamAB*, *mamCD*, and *mms6* clusters, are nonmagnetic and cannot form a magnetosome membrane (47, 56, 57).

### The Magnetosome Island

The combination of these proteomic and genetic results, as well as comparisons between the genome sequences of various MB, has shown that the majority of magnetosome genes are clustered within an unstable genomic island, commonly referred to as the magnetosome island (MAI) (57). The putative MAI of MSR-1 contains hallmarks of mobile genomic islands, such as pseudogenes and insertion sequence elements. The large number of insertion sequence elements seems to make this region extremely unstable, and various insertions, deletions, and rearrangements...
within the MAI have been observed in MSR-1 cultures subjected to stress conditions, such as extended cold storage and oxidative stress (57). A specific mechanism for the occurrence of MAI deletions has been proposed for AMB-1 (47). In this organism, a 98-kb genomic region surrounding the mamAB, mamCD, and mms6 gene clusters is marked by two 1132-bp direct repeats, and a putative integrase gene can be found near one of these repeats. Polymerase chain reaction analysis of AMB-1 cultures revealed the presence of a circular DNA intermediate of a possible integrase-mediated excision of the MAI (47). Because the MAI constitutes \( \sim 2\% \) of the entire genome sequence of the bacterium, there may be a competitive advantage for cells that can selectively delete this genomic region. Also, the presence of the excised MAI as a circular intermediate supports the idea that magnetosome formation may be transferred laterally between various organisms.

The MAI of MSR-1 and AMB-1 displays many shared characteristics. For instance, half of the genes of the AMB-1 MAI have a homolog in MSR-1, and the organization of the major gene clusters is highly similar between the two organisms (47, 57). However, their differences are also quite significant. The relative positions and orientations of the conserved regions, such as the mam and mms gene clusters, differ significantly between the two organisms. Also, regions of the AMB-1 MAI that are conserved in the Magnetospirillum magnetotacticum sp. MS-1 genome are absent from the MSR-1 MAI (47, 57). The role of the MAI genes not shared between MSR-1 and AMB-1 is unclear. They might be “junk” DNA without a specific role in magnetosome formation, or they may determine the variable features of magnetosomes, such as the species-specific control over the number of magnetosomes, the number of chains, and the process of chain assembly.

The above results highlight the importance of the genes within the MAI in magnetosome formation. But are they sufficient for this process to occur? Both of the proteomic studies mentioned above identified proteins that are associated with magnetosomes but encoded by genes outside the MAI (40, 51). Some of these are certain to be contaminants from very abundant cellular proteins or other cellular structures copurifying with the magnetosomes. Others, however, could be dedicated magnetosome proteins encoded by non-MAI genes. Additionally, in a recent genetic analysis of AMB-1, none of the 69 transposon insertions resulting in nonmagnetic cells were found to be within the MAI (51). This stands in contrast to a previously published report that all mutants had insertions in the mamAB gene cluster (31). It is possible that the choice of transposons or screening methods used by these two groups explains the differences between the results of the screens. In addition, given the reported occurrence of spontaneous MAI deletions in AMB-1, it is important that all mutants in these studies are analyzed further to ensure the presence of this genomic region. One way to reconcile these inconsistent results is to assume that the MAI is sufficient for the unique aspects of magnetosome formation and that other cellular pathways, common to all bacteria, can regulate the various steps of this process. A prediction of such a scenario is that the introduction of the MAI into non-MB should lead to magnetosome formation.

**FUNCTIONAL ANALYSIS OF MAGNETOSOME GENES**

The identification of the MAI represents a major step forward in elucidating the molecular basis of magnetosome formation. The genes of the MAI (and perhaps a few other genes) represent a large parts list for production of a functioning organelle, but their exact functions remain unknown. The primary sequence of most MAI-encoded proteins is not useful in predicting their role in magnetosome formation. Approximately half of these proteins have no homologs with known
functions in other organisms, and for others, only a general function can be predicted. For example, MamE is predicted to be an HtrA-like protease, and MamB belongs to the ubiquitous cation diffusion facilitator family of transporters. Clearly, detailed molecular studies at the genetic and biochemical level are required to determine the specific role of these genes in the various steps required for magnetosome formation. A few recent studies, which have focused on specific genes within the MAI, have shed light also on their possible functions.

**mamCD and mms6 Operons**

In many organisms, the minerals produced through biomineralization are often intimately associated with the proteins controlling their shape and size (58, 59). It now appears that the same is true for the magnetic minerals of MB. In an intriguing set of experiments, Arakaki et al. (60) demonstrated that among the many magnetosome membrane proteins a small subset was tightly associated with the magnetite crystals of AMB-1. These four proteins, Mms5, Mms6, Mms7, and Mms13 (MamG, Mms6, MamD, and MamC in MSR-1), were not extracted from magnetosomes by a urea, and detergent treatment, which removed the membrane and most other proteins, could only be solubilized by boiling in a sodium dodecyl sulfate solution (60). They are encoded by the genes of the **mamCD** and **mms6** gene clusters, which appear adjacent to each other in both the AMB-1 and MSR-1 MAIs, confirming their potential role in magnetosome formation. In a separate study, T aoka et al. (61) followed the in vivo localization of Mam12, the *Magnetospirillum magnetotacticum* sp. MS-1 homolog of Mms13 and MamC, using immunogold labeling and TEM. In these studies, Mam12 had a much closer association with the magnetite crystal than another magnetosome membrane protein, thus supporting the results of the biochemical experiments. Arakaki et al. also demonstrated that recombinant Mms6 could bind iron and that its presence in an in vitro magnetite synthesis reaction resulted in particles with similar size and shape to those produced within magnetosomes (60). Mms5, Mms6, and Mms7 all contain a hydrophobic peptide domain, containing repeats of glycine and leucine not found in other bacteria (60). However, large stretches of similar glycine-leucine repeats are seen in silk fibroin-like proteins that are known to be involved in formation of calcium-based minerals in other systems, thus providing a potential evolutionary link between a bacterial biomineralization system and those found in eukaryotes.

**mamA**

One of the primary pieces of evidence for the involvement of the **mamAB** gene cluster in magnetosome formation has been that transposon insertions within it result in nonmagnetic cells (31). The organization of genes within the **mamAB** cluster suggests that it could be an operon, making it likely that the transposon mutants are defective in the expression of all genes downstream of the insertion site. To combat this issue, some of the **mamAB** cluster genes have now been directly analyzed through the generation of nonpolar deletion mutants. A mutant of the **mamA** gene, encoding a tetratricopeptide repeat domain protein known to associate with magnetosomes (52, 55), had no apparent defect in biomineralization, and the kinetics of crystal formation as well as the final size and shape of individual magnetite crystals were similar to wild-type cells (31). However, the Δ*mamA* mutants produced only half the number of magnetite crystals per cell when compared to wild-type AMB-1. This defect was not due to the absence of the magnetosome membrane because the mutant had a wild-type pattern and number of “empty” magnetosome membranes (31).

The subcellular localization of MamA has also been examined by a variety of methods.
MreB: a bacterial actin-like protein known to act as a cytoskeletal element in various bacterial species

Fusions of MamA to the green fluorescent protein were used to monitor its localization dynamics in live cells (31). MamA localized as a patchy line within the cell during an exponential growth phase and as a few punctate spots within the cell in a stationary phase. Because MamA does not appear to contain a signal sequence or any transmembrane domains, it is likely that it localizes to the cytoplasmic side of the magnetosome membrane. This idea was confirmed in immunogold TEM experiments with Mam22, the Magnetospirillum magnetotacticum sp. MS-1 homolog of MamA, whereby the protein was shown to be peripherally associated with magnetosomes in the cytoplasm (61).

What might be a possible role for MamA given the above results? The “short chain” phenotype of ΔmamA mutants has been observed previously for wild-type strains of MB when varying environmental conditions resulted in changes in the length of the magnetosome chain (62–64). In fact, the length of the magnetosome chain differs even among closely related MB species. One possibility then is that MamA participates in signaling events that dictate how many magnetosome membranes within a chain can be activated for magnetite biomineralization under a given environmental condition. Tetra-tricopeptide repeat domains are found in many different proteins, as well as in a variety of organisms, and are thought to promote protein-protein interactions (65). As such, MamA may not have a specific enzymatic activity but may act as a scaffold linking cytoplasmic proteins to the magnetosome membrane.

mamJ and mamK

Genetic studies of two other MAI genes, mamJ and mamK, have revealed a role for them in the formation and stability of the magnetosome chain (30, 32). MamJ is a protein unique to MB that has been shown to interact with the magnetosome membrane (40). Similar to some biomineralization proteins, MamJ contains large repeats of acidic amino acids, implying a potential role in formation of magnetite crystals (59). But in a deletion of the mamJ gene in MSR-1 magnetite biomineralization was not affected. Instead, magnetosomes failed to form a chain and collapsed into aggregates within the cytoplasm (32). In ECT reconstructions, it was clear that structures resembling the magnetosome-associated cytoskeleton still existed in the ΔmamJ mutants, but magnetosomes were no longer interacting with these filaments (32). The conclusion drawn from these experiments is that MamJ mediates the interaction between the magnetosome and the magnetosome-specific cytoskeletal filaments.

The potential identity of these filaments was revealed in a detailed examination of mamK, an MAI gene located immediately downstream of mamJ (30). MamK, which has also been shown to interact with magnetosomes (51), is a homolog of MreB, the bacterial actin-like protein implicated in cell shape determination, establishment of cell polarity, and chromosome segregation (33). MreB can polymerize into filaments in vitro, and its crystal structure bears a striking resemblance to that of eukaryotic actin (34). Similar to MreB and its other homologs, MamK fusions to green fluorescent protein displayed a filament-like organization in vivo, appearing as a thin line within the cell (30). The formation of filament-like structures appears to be an intrinsic property of the protein because MamK can form filaments in E. coli cells as well (66). In addition, the filaments of the magnetosome-specific cytoskeleton, as visualized by ECT imaging, had dimensions similar to MreB filaments produced in vitro (30). These observations led to the hypothesis that MamK might be the structural element of the magnetosome cytoskeleton with a role in the organization of the magnetosome chain. Accordingly, strains deleted for mamK had no defects in production of magnetosome membrane invaginations or magnetite biomineralization, but the magnetosomes lost their chain-like structure and were dispersed.
throughout the cell (30). In addition, no filaments were near the magnetosomes of the \( \Delta \text{mamK} \) strain, implying that the magnetosome cytoskeleton is either composed of or regulated by MamK (30).

The simplest model derived from the above studies is that MamJ mediates the interaction between magnetosomes and their dedicated cytoskeleton, likely to be composed of MamK. The phenotypes of the two mutants, however, are strikingly different from each other (e.g., aggregated magnetosomes in \( \Delta \text{mamJ} \) mutant and dispersed magnetosomes in the \( \Delta \text{mamK} \) mutant), implying that the two proteins may not interact. By contrast, because the mutants were generated in two distinct strains, it is possible that MamK and MamJ interact but that the phenotypic differences are due to species-specific modes of chain formation (see above). The exact function of MamK and the magnetosome cytoskeleton is not readily discernible from these genetic studies. MamK might be involved in establishing the chain by bringing newly formed magnetosomes into the magnetosome chain. In the absence of MamK, individual magnetosomes will then disperse throughout the cell membrane (Figure 3). Alternatively, MamK might play a role in maintaining the structure of the magnetosome chain after it has already been established. For instance, MamK might prevent membrane synthesis within the chain during cell growth, and in its absence, lipids might be inserted between the magnetosomes of a preexisting chain, thus pushing them apart from each other (Figure 3). Regardless of the specific function of MamK, these studies show that, just like eukaryotes, bacteria can use cytoskeletal proteins for controlling organelle positioning and dynamics (67).

MODEL FOR MAGNETOSOME FORMATION

The combination of physical and molecular studies detailed in this review leads to a simple model for magnetosome formation (Figure 4). Because the magnetosome membrane preexists magnetite formation and is the site of its biomineralization, the first step in this process is the biogenesis of the magnetosome membrane and the targeting of magnetosome proteins to this compartment. The proteins responsible for producing the magnetosome membrane have not been identified yet. However, it is likely that some of the genes involved in this step are present in the MAI because a deletion of this region leads to a failure in magnetosome membrane biogenesis (A. Komeili, unpublished results). Similar to membrane trafficking in eukaryotes, protein sorting to the magnetosome membrane might occur concurrently with the invagination of the cell membrane (68). However, as magnetosomes are continuous with the cell membrane, there is an opportunity to transport magnetosome proteins to the membrane after it has been formed, possibly via a “diffusion and capture” mechanism observed in other bacterial systems (69). Once a magnetosome membrane invagination has been formed, it has to be assembled into a chain with the help of the MAI-encoded proteins, MamK and MamJ. Both of these proteins interact with the magnetosome membrane but are not predicted to have transmembrane
Cell cycle
Stress
Environment

Cell membrane proteins

Magnetosome proteins

Membrane biogenesis & protein sorting

Chain formation

mamJ
mamK

Biomineralization

mms6 operon
mamCD operon

Figure 4
Model for magnetosome formation. Magnetosomes are formed in three steps. First, a membrane invagination is derived from the inner membrane, and magnetosome proteins (green) are sorted away from cell membrane proteins (orange). Second, individual invaginations are assembled into a chain with the help of MamJ and MamK proteins. Third, iron is transformed into highly ordered magnetite crystals within the magnetosome membrane with the possible involvement of genes from the mamCD and mms6 operons. Cell cycle, environmental conditions, and cellular stress may feed in at any of the three steps to modulate the formation of magnetosomes.

CONCLUDING REMARKS
In his 1982 Annual Review of Microbiology article, Blakemore stated that his “purpose is not only to critically discuss available information, but also, hopefully, to spark in some readers interest and activity in this new area of research” (4). In many ways, this wish has come true with magnetosome research spanning disciplines as diverse as geobiology and biotechnology. The rapid progress made in identifying a large number of magnetosome proteins is likely that other proteins are involved in magnetosome chain formation. The final step in this model is the biomineralization of magnetite, which encompasses all the reactions involving iron, including its uptake from outside of the cell, oxidation to ferrihydrite in the periplasm, transport into the magnetosome, and partial reduction to form magnetite. It also includes the control of shape and size, possibly through the gene products of the mms6 and mamCD operons. Other processes, such as environmental influences on magnetite synthesis and magnetosome chain length as well as the cell cycle regulation of magnetosome formation, can feed in at different steps of this model. As is clear from the model above, the genes of the MAI are involved at various steps of magnetosome formation, including membrane biogenesis, chain formation, and biomineralization, making this region a logical target for future magnetosome research.
genes has laid a solid foundation for defining the molecular mechanisms leading to the formation of this organelle. Hopefully, this review helps introduce biochemists and cell biologiststo the tremendous potential of this young field and, in the process, inspires some to join the small but growing list of magnetosome researchers.

**SUMMARY POINTS**

1. Magnetosomes are membranous organelles of MB sharing many of the features of eukaryotic organelles. The magnetosome membrane is an invagination of the cell membrane, contains a distinct set of proteins, and is organized via a dedicated cytoskeleton.

2. Most magnetosome-specific proteins are encoded by genes organized within a discrete genomic region termed the magnetosome island (MAI). These genes have been implicated in the formation of the magnetosome membrane, organization of the magnetosome chain, and biomineralization of magnetite, raising the possibility that the MAI contains all the unique genes required for making a magnetosome chain.

3. Many of the proteins encoded by the *mamCD* and *mms6* operons are tightly associated with the magnetite crystal and contain a Leu-Gly-rich domain related to silk fiber proteins. One of these proteins, Mms6, can bind iron and influence the shape of magnetite crystals produced in vitro.

4. The actin-like protein MamK and the acidic protein MamJ are required for the proper organization of the magnetosome chain. MamK is needed for the formation of a magnetosome-specific cytoskeleton and might be its structural component. MamJ is proposed to mediate the interaction between magnetosomes and MamK.

**FUTURE ISSUES**

1. What is the real function of magneto-aerotaxis? Are the south-seeking MB in the Northern Hemisphere an anomaly or a clue into the real function of magneto-aerotaxis? Are there any Archea or gram-positive bacteria capable of magnetosome formation? What evolutionary mechanisms account for the widespread presence of magneto-aerotaxis across the phylogenetic tree?

2. Do the proteins tightly associated with magnetite (such as Mms6) control crystal shape formation in vivo? What accounts for the species-specific pattern to crystal shape and size? Is magnetite biomineralization related to other known biomineralization processes?

3. What are the molecular mechanisms of membrane invagination and protein sorting to the magnetosome? Are they related to formation of membranous organelles in other bacteria or eukaryotes?

4. Is the magnetosome cytoskeleton responsible for establishment or maintenance of the magnetosome chain? Is MamK a structural component of the magnetosome cytoskeleton and does it interact with MamJ? Is the cytoskeleton dynamic? And do other proteins regulate its polymerization?
ACKNOWLEDGMENTS

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**Errata**

An online log of corrections to *Annual Review of Biochemistry* chapters (if any, 1997 to the present) may be found at http://biochem.annualreviews.org/errata.shtml